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L7: Entry 2 of 11 File: USPT Sep 25, 2001

DOCUMENT-IDENTIFIER: US 6293970 B1
TITLE: Plasticized bone and soft tissue grafts and methods of making and using same

Brief Summary Paragraph Right (5):
Bone tissue in the form of bone grafts for implantation into a patient, is typically preserved and provided in a dehydrated state. Dehydration of bone tissue through drying, whether by air drying or sublimation as in freeze-drying, results in alteration of the molecular structure of the bone tissue and as a result of the reorientation of the collagen fibrils and the crystalline mineral phase, stress accumulates in the bone tissue. This stress can be relieved by rehydration or by the occurrence of small or large dislocations of structure. Small dislocations are designated micro fractures and are not usually visible to the naked eye. Large dislocations are designated fractures and are usually visible to the naked eye.

Brief Summary Paragraph Right (6):
In a long bone, for example a femur, tibia, fibula, or humerus, the shaft separates the proximal and distal ends of the long bone. The shaft serves to focus loads applied to the whole bone into a smaller diameter than found at the proximal and distal ends of the long bone and the shaft of a long bone is typically of a cylindrical shape and is comprised of compact (cortical) bone. Loads applied along the axis of the shaft require that the cortical bone maintain a constant circumference, i.e. the tendency to failure would distort the bone tissue perpendicular to the axis of load application. Thus, the orientation of the collagen fibers should be such that tensile stress is resisted along the axis of loading and compressive stress is resisted perpendicular to loading. Drying of shaft portions of long bones results in reorientation of collagen fibers and the mineral phase such that changes in the circumferential orientation create stress within the bone matrix which can be relieved only by rehydration or occurrence of a fracture which allows a reorientation approximating the original orientation. In dehydrated cortical ring grafts cut from shafts of long bones, this stress release can present as a fracture along the long axis of the bone shaft leaving a circumference which approximates the circumference of the cortical ring graft prior to drying. By rehydrating bone grafts prior to implantation, the potential for fracture formation which can compromise the function of the bone product can be reduced, but not eliminated. Fractures as discussed above can occur in dehydrated bone prior to rehydration and result in a graft having compromised biomechanical properties, which in turn can result in graft failure when implanted in a patient.

Brief Summary Paragraph Right (56):
Bone tissue is cleaned and processed as described in U.S. Pat. No. 5,556,379, and co-pending U.S. patent application Ser. Nos. 08/871,601; 08/620,858; 08/646,520; and 08/646,519 by for example, transection of an essentially intact bone or perforation of an essentially intact bone with attachment of sterile plastic tubing to the cut end of a transected bone or to an attachment port inserted into the perforation of the perforated bone. The bone is immersed in a cleaning solution, such solutions including known cleaning agents as well as those described in the above-identified patent and co-pending patent applications, with or without use of sonication. The cleaning solution is induced to flow into, through, and out of the bone through use of a peristaltic pump or negative pressure applied to the cleaning solution. The induced flow of cleaning solution draws the bone marrow from the interior of the bone, and particularly from the cancellous bone marrow space, where it can be safely deposited in a receiving container containing a strong virucidal agent such as sodium

hypochlorite (common bleach). The cleaned bone can then be further cleaned by causing the cleaning solution to be replaced with a solution of one or more decontaminating agents, including for example 3% hydrogen peroxide, with or without plasticizer. Hydrogen peroxide which in addition to its mild disinfection activity generates oxygen bubbles that can further assist in dislodging residual bone marrow materials causing the residual bone marrow materials to flow from the bone and into the receiving container.

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L1: Entry 4 of 13 File: USPT Nov 30, 1982

DOCUMENT-IDENTIFIER: US 4361697 A
TITLE: Extraction, separation and recovery of diterpene glycosides from Stevia rebaudiana plants

Detailed Description Paragraph Right (10):
In the foregoing preferred mode of operation, the second solvent, i.e., the solvent employed in the second extraction of the plant material, is removed from the extracted DTG materials and replaced by the preferred third solvent of lower polarity in a suitable manner, such as by evaporation to dryness under pressure and temperature conditions which avoid degradation of the materials, followed by dissolution of the residue in the third solvent. It has been found preferable for substantially complete dissolution of the residue to add a small minor amount of water to the preferred 1-propanol, and, in particular, to provide a solvent of 95-100% by volume of 1-propanol and the balance water. The residue is dissolved in a conventional manner, such as by stirring with the solvent at ambient temperature or with warming. The quantity of solvent required to dissolve the residue will depend upon the composition of the particular plant material subjected to extraction and thus cannot be predicted beforehand since, as is true of many natural materials, this composition is not invariable.

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L7: Entry 6 of 11 File: USPT Aug 11, 1992

DOCUMENT-IDENTIFIER: US 5138030 A
TITLE: Process for extracting type I collagen form an avian source, and applications therefor

Brief Summary Paragraph Right (19):
The avian source from which type I collagen is extracted is preferably feet from freshly slaughtered poultry (i.e., less than 8 hours from slaughter). The avian feet are washed thoroughly with tap water (temperature less than 70.degree. F.), until all feathers and surface debris are removed. In order to remove bacterial contaminants, the avian feet are then soaked in 50-100 ppm of sodium hypochlorite at pH 7-8, with agitation. Then the avian feet are rinsed thoroughly with pyrogen-free water.

Brief Summary Paragraph Right (20):
The cleaned and decontaminated feet are then ground, using a commercially available grinder for this purpose. The comminuted avian feet are again washed with 50-100 ppm of sodium hypochlorite at pH 7-8, with agitation. An optional wash with 70% ethanol/30% pyrogen-free water may follow if the pyrogen load of the material is high.

Detailed Description Paragraph Center (11):
Crosslinking of the Collagen Matrix by Heat and Dehydration

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L3: Entry 1 of 3 File: USPT May 7, 1996

DOCUMENT-IDENTIFIER: US 5514811 A
TITLE: Process for synthesizing 4-halo-5-(hydroxymethyl) imidazole compounds

Detailed Description Paragraph Right (19):
11.5 Grams (15 mmol) of an aqueous solution containing 9.7% of sodium hypochlorite was dropwisely added to a solution consisting of 2.53 g (16.2 mmol) of the 2-ethyl-4,5-bis(hydroxymethyl) imidazole and 50 ml of water. After the dropwise addition has been finished, the mixture was stirred at room temperature for one hour, followed by neutralization by the addition of dry ice and further followed by evaporation to dryness under reduced pressure. The resulting solid was extracted with ethanol, the extract was evaporated under reduced pressure, and the resulting solid product was extracted with acetone. 1.48 Grams of the starting 2-ethyl-4,5-bis(hydroxymethyl) imidazole was recovered as an extraction residue, the extract was concentrated under reduced pressure, and was subjected to the column chromatography (silica gel-acetone) to obtain a 2-ethyl-4-chloro-5-(hydroxymethyl) imidazole in an amount of 0.46 g (yield 43.0% with respect to the starting material consumed). The TLC and spectra were measured to be the same as those of Example 3.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 8. Document ID: JP 2000297044 A

L1: Entry 8 of 13 File: JPAB Oct 24, 2000

PUB-NO: JP02000297044A
DOCUMENT-IDENTIFIER: JP 2000297044 A
TITLE: TRANSDERMAL PATCH FOR EXTERNAL USE FOR SKIN

PUBN-DATE: October 24, 2000

INVENTOR-INFORMATION:
NAME COUNTRY
NAGAO, TAMOTSU
MORITA, NAOKI

INT-CL (IPC): A61 K 35/78; A61 K 7/00; A61 K 9/70

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 9. Document ID: JP 2000159657 A

L1: Entry 9 of 13 File: JPAB Jun 13, 2000

PUB-NO: JP02000159657A
DOCUMENT-IDENTIFIER: JP 2000159657 A
TITLE: COSMETIC

PUBN-DATE: June 13, 2000

INVENTOR-INFORMATION:
NAME COUNTRY
TANAKA, HIROSHI

INT-CL (IPC): A61 K 7/48; A61 K 7/00; A61 P 17/00; A61 K 35/78

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 10. Document ID: KR 235685 B1, EP 685235 A1, AU 9467517 A, JP 07330788 A, FI 9403295 A, CA 2127762 A, ES 2081782 T1, US 5635185 A, IT 1269880 B, AU 684781 B, EP 685235 B1, DE 69422980 E, ES 2081782 T3

L1: Entry 10 of 13 File: DWPI Feb 1, 2000

DERWENT-ACC-NO: 1996-012142
DERWENT-WEEK: 200118

COPYRIGHT 2002 DERWENT INFORMATION LTD
TITLE: Prepn of extracts of Piliostigma thonningii - which are useful in treatment of viral infections.

INVENTOR: BOMBARDELLI, E; MORAZZONI, P ; MUSTICH, G

PRIORITY-DATA: 1994IT-MI01135 (June 1, 1994)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
KR 235685 B1	February 1, 2000		000	A61K035/78
EP 685235 A1	December 6, 1995	E	007	A61K035/78
AU 9467517 A	January 4, 1996		000	A61K035/78
JP 07330788 A	December 19, 1995		005	C07G017/00
FI 9403295 A	December 2, 1995		000	A61K035/78
CA 2127762 A	December 2, 1995		000	A61K035/78
ES 2081782 T1	March 16, 1996		000	A61K035/78
US 5635185 A	June 3, 1997		006	A61K035/78
IT 1269880 B	April 15, 1997		000	A61K000/00
AU 684781 B	January 8, 1998		000	A61K035/78
EP 685235 B1	February 9, 2000	E	000	A61K035/78
DE 69422980 E	March 16, 2000		000	A61K035/78
ES 2081782 T3	July 1, 2000		000	A61K035/78

INT-CL (IPC): A61 K 0/00; A61 K 31/685; A61 K 35/78; A61 K 47/24; C07 G 11/00; C07 G 17/00

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 11. Document ID: FR 2465484 A

L1: Entry 11 of 13 File: DWPI Apr 24, 1981

DERWENT-ACC-NO: 1981-42489D
DERWENT-WEEK: 198124
COPYRIGHT 2002 DERWENT INFORMATION LTD
TITLE: Extract of e.g. eugenia jambolana lamarck - has antidiabetic, hypoglycaemi c, hypolipaemic, weight reducing anorexic and anti-cataract activity

INVENTOR: RATSIMAMAN, S

PRIORITY-DATA: 1979FR-0023590 (September 21, 1979)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
FR 2465484 A	April 24, 1981		000	

INT-CL (IPC): A61K 35/78

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 12. Document ID: FR 2456747 A

L1: Entry 12 of 13 File: DWPI Jan 16, 1981

DERWENT-ACC-NO: 1981-16050D
DERWENT-WEEK: 198110
COPYRIGHT 2002 DERWENT INFORMATION LTD
TITLE: Extn. of anthocyanoside(s) e.g. malvoside or cyanoside - with acidified alcohol, drying by azeotropic distn., redissolving in acidified alcohol and prepn. with ketone(s)

PRIORITY-DATA: 1979FR-0012573 (May 17, 1979)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
FR 2456747 A	January 16, 1981		000	

INT-CL (IPC): A61K 31/70; C07H 17/06; C09B 61/00

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 13. Document ID: FR 2292485 A, GB 1513158 A

L1: Entry 13 of 13 File: DWPI Jul 30, 1976

DERWENT-ACC-NO: 1976-76231X
DERWENT-WEEK: 197641
COPYRIGHT 2002 DERWENT INFORMATION LTD
TITLE: Dried aq. extract of Senecio - esp. groundsel and ragwort, with antiulcer activity

PRIORITY-DATA: 1974GB-0051879 (November 29, 1974)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
FR 2292485 A	July 30, 1976		000	
GB 1513158 A	June 7, 1978		000	

INT-CL (IPC): A61K 31/70; A61K 35/78; C07D 309/10

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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Terms	Documents
(evaporation adj2 dryness) same (plant or animal) same (extract$)	13

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Your wildcard search against 2000 terms has yielded the results below

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Search Results - Record(s) 1 through 13 of 13 returned.

☐ 1. Document ID: US 5403852 A

L1: Entry 1 of 13 File: USPT Apr 4, 1995

US-PAT-NO: 5403852
DOCUMENT-IDENTIFIER: US 5403852 A

TITLE: Oxazole derivatives, their preparation and pharmaceutical compositions containing them

DATE-ISSUED: April 4, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barreau; Michel	Montgeron			FRX
Kryvenko; Michel	Paris			FRX
Lavergne; Marc-Pierre	Mandres les Roses			FRX
Techer; Auguste	Avon			FRX

US-CL-CURRENT: 514/374; 548/235, 548/236

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 4952603 A

L1: Entry 2 of 13 File: USPT Aug 28, 1990

US-PAT-NO: 4952603
DOCUMENT-IDENTIFIER: US 4952603 A

TITLE: Method for the isolation of artemisinin from Artemisia annua

DATE-ISSUED: August 28, 1990

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
ElFeraly; Farouk S.	Oxford	MS	38655	
ElSohly; Hala N.	Oxford	MS	38655	

US-CL-CURRENT: 514/450

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC
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☐ 3. Document ID: US 4565700 A

L1: Entry 3 of 13 File: USPT Jan 21, 1986

US-PAT-NO: 4565700
DOCUMENT-IDENTIFIER: US 4565700 A

TITLE: Process for preparation of substance having hypotensive component

DATE-ISSUED: January 21, 1986

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Suzuki; Tetsuya	Ayase-shi, Kanagawa 252			JPX

US-CL-CURRENT: 424/757

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC
Draw Desc | Image

☐ 4. Document ID: US 4361697 A

L1: Entry 4 of 13 File: USPT Nov 30, 1982

US-PAT-NO: 4361697
DOCUMENT-IDENTIFIER: US 4361697 A

TITLE: Extraction, separation and recovery of diterpene glycosides from Stevia rebaudiana plants

DATE-ISSUED: November 30, 1982

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dobberstein; Robert H.	Wheaton	IL		
Ahmed; Mohamed S.	Cairo			EGX

US-CL-CURRENT: 536/128; 536/127

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 5. Document ID: US 4235890 A

L1: Entry 5 of 13 File: USPT Nov 25, 1980

US-PAT-NO: 4235890
DOCUMENT-IDENTIFIER: US 4235890 A

TITLE: Stephania cepharantha extract, its method of preparation and its use as pharmaceutical

DATE-ISSUED: November 25, 1980

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Debat; Jacques	Saint Cloud			FRX
Lemoine; Jean	Garches			FRX
Gabillault; Francoise L.	Plaisir			FRX

US-CL-CURRENT: 424/779

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 6. Document ID: US 4154826 A

L1: Entry 6 of 13 File: USPT May 15, 1979

US-PAT-NO: 4154826
DOCUMENT-IDENTIFIER: US 4154826 A

TITLE: Thiophosphorylguanidines for combating pests

DATE-ISSUED: May 15, 1979

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rathgeb; Paul	Basel			CHX

US-CL-CURRENT: 514/112; 514/115, 514/118, 558/167, 558/171, 558/176, 987/201

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 7. Document ID: US 3948911 A

L1: Entry 7 of 13 File: USPT Apr 6, 1976

US-PAT-NO: 3948911
DOCUMENT-IDENTIFIER: US 3948911 A

TITLE: Substituted quinoxaline-2-carboxamide 1,4-dioxides

DATE-ISSUED: April 6, 1976

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
McFarland; James W.	Lyme	CT		

US-CL-CURRENT: 544/355; 544/116, 544/62, 548/126, 552/8

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L1: Entry 10 of 13 File: DWPI Feb 1, 2000

DERWENT-ACC-NO: 1996-012142
DERWENT-WEEK: 200118
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TITLE: Prepn of extracts of Piliostigma thonningii - which are useful in treatment of viral infections.

Basic Abstract Text:
The following are claimed: (I) Prepn of extracts from Piliostigma thonningii, comprising extracting all parts of the plant, separately or mixed together with low mol. wt. aliphatic alcohols or ketones (pure or diluted with water), then subjecting the resulting extracts to a treatment comprising: (a) elimination of the solvent by concn at below 40deg.C; (b) filtration or centrifugation to remove the gummy residues of undesired substances formed during the elimination of the organic solvent; (c) extn of the clear aq extract with EtOAc to complete exhaustion in the extractable substances and elimination of the organic extracts; (d) chromatography of the aq phase on an adsorption resin column, and re-elution with aliphatic low mol wt alcohols or ketones diluted with water; (e) partial concn of the eluate from the column under vacuum and at temps not above 60 deg.C; (f) evapn of the concentrate to dryness under vacuum; (g) dissolution of the residue in a small volume of MeOH; and (h) tre atment of the soln with an amt of CH2Cl2 sufficient to ppte the active ingredient.

Equivalent Abstract Text:
The following are claimed: (I) Prepn of extracts from Piliostigma thonningii, comprising extracting all parts of the plant, separately or mixed together with low mol. wt. aliphatic alcohols or ketones (pure or diluted with water), then subjecting the resulting extracts to a treatment comprising: (a) elimination of the solvent by concn at below 40 deg. C; (b) filtration or centrifugation to remove the gummy residues of undesired substances formed during the elimination of the organic solvent; (c) extn of the clear aq extract with EtOAc to complete exhaustion in the extractable substances and elimination of the organic extracts; (d) chromatography of the aq phase on an adsorption resin column, and re-elution with aliphatic low mol wt alcohols or ketones diluted with water; (e) partial concn of the eluate from the column under vacuum and at temps not above 60 deg. C; (f) evapn of the concentrate to dryness under vacuum; (g) dissolution of the residue in a small volume of MeOH; and (h) tre atment of the soln with an amt of CH2Cl2 sufficient to ppte the active ingredient.

Basic Abstract Text (1):
The following are claimed: (I) Prepn of extracts from Piliostigma thonningii, comprising extracting all parts of the plant, separately or mixed together with low mol. wt. aliphatic alcohols or ketones (pure or diluted with water), then subjecting the resulting extracts to a treatment comprising: (a) elimination of the solvent by concn at below 40deg.C; (b) filtration or centrifugation to remove the gummy residues of undesired substances formed during the elimination of the organic solvent; (c) extn of the clear aq extract with EtOAc to complete exhaustion in the extractable substances and elimination of the organic extracts; (d) chromatography of the aq phase on an adsorption resin column, and re-elution with aliphatic low mol wt alcohols or ketones diluted with water; (e) partial concn of the eluate from the column under vacuum and at temps not above 60 deg.C; (f) evapn of the concentrate to dryness under vacuum; (g) dissolution of the residue in a small volume of MeOH; and (h) tre atment of the soln with an amt of CH2Cl2 sufficient to ppte the active

ingredient.

Equivalent Abstract Text (1):
The following are claimed: (I) Prepn of extracts from Piliostigma thonningii, comprising extracting all parts of the plant, separately or mixed together with low mol. wt. aliphatic alcohols or ketones (pure or diluted with water), then subjecting the resulting extracts to a treatment comprising: (a) elimination of the solvent by concn at below 40 deg. C; (b) filtration or centrifugation to remove the gummy residues of undesired substances formed during the elimination of the organic solvent; (c) extn of the clear aq extract with EtOAc to complete exhaustion in the extractable substances and elimination of the organic extracts; (d) chromatography of the aq phase on an adsorption resin column, and re-elution with aliphatic low mol wt alcohols or ketones diluted with water; (e) partial concn of the eluate from the column under vacuum and at temps not above 60 deg. C; (f) evapn of the concentrate to dryness under vacuum; (g) dissolution of the residue in a small volume of MeOH; and (h) tre atment of the soln with an amt of CH2Cl2 sufficient to ppte the active ingredient.

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L7: Entry 5 of 11 File: USPT Sep 29, 1998

DOCUMENT-IDENTIFIER: US 5814328 A
TITLE: Preparation of collagen using papain and a reducing agent

Abstract Paragraph Left (1):
Purified collagen is produced by contacting a collagen sample with a first proteolytic enzyme, a reducing agent and a second proteolytic enzyme. Preferably, the first and second proteolytic enzymes are papain and the reducing agent is sodium sulfide, dithiothreitol, glutathionine or sodium borohydride. The purified collagen may be contacted with a delipidation agent such as chloroform or methanol and with a phosphorylation agent such as sodium trimetaphosphate to form a biocompatible collagen. Prior to phosphorylation, the delipidated collagen may be treated by compressing, dehydrating, dispersing and drying to form collagen fibers. Also, prior to phosphorylation, the delipidated collagen may be subjected to filter-sterilizing. De-epithelializing of the collagen may carried out prior to treating with the first proteolytic enzyme. The purified and biocompatible collagen may be used in transplantation or hemostasis, and may be provided with compounds such as antimicrobials, antivirals, growth factors and other compounds suitable for biomedical use.

Brief Summary Paragraph Right (15):
Collagen preparations are typically prepared from skin, tendons (e.g., bovine Achilles, tail, and extensor tendons), hide or other animal parts, by procedures involving acid and/or enzyme extraction. Basically, collagen preparation methods involve purification of collagen by extraction with diluted organic acids, precipitation with salts, optional gelation and/or lyophilization, tangential filtration etc. After separating facia, fat and the impurities, the tissue is subjected to moderate digestion with proteolytic enzymes, such as pepsin, then the collagen is precipitated at a neutral pH, redissolved and the residual impurities precipitated at an acid pH. The tissue is then digested with a strong alkali and then exposed to acid to facilitated swelling. The collagen fibers are then precipitated with salts or organic solvents, and dehydrating the collagen fibers. (See e.g., U.S. Pat. No. 5,028,695, herein incorporated by reference). Eventually the extracted collagen can be converted into a finely divided fibrous collagen by treating water-wet collagen with acetone to remove water, centrifuging to obtain the solid mass of collagen and deaggregating the collagen during drying. (See e.g., U.S. Pat. No. 4,148,664, herein incorporated by reference). The collagen preparation can then be brought back to a neutral pH and dried in the form of fibers. Completely transparent, physiological and hemocompatible gels, collagen films, and solutions can be prepared. These forms of collagen may then be used in the fabrication of contact lenses and implants.

Brief Summary Paragraph Right (26):
In a particularly preferred embodiment, the method of the present invention further comprises the steps of compressing the delipidated collagen to produce compressed collagen; dehydrating the compressed collagen to produce dehydrated collagen; and dispersing and drying the dehydrating collagen to form collagen fibers. Thus, in this embodiment of the methods of the present invention, the collagen fibers are dried.

Brief Summary Paragraph Right (29):
It is further contemplated that the purified collagen be comprised of additional compounds, including but not limited to antimicrobials, antivirals, growth factors, anti-dehydration compounds, antiseptics, or other compounds suitable for biomedical

and/or veterinary uses.

Brief Summary Paragraph Right (35):
In yet a further embodiment, the method of the present invention further comprises the steps of compressing the delipidated collagen to produce compressed collagen; dehydrating the compressed collagen to produce dehydrated collagen; and dispersing and drying the dehydrating collagen to form collagen fibers. Thus, in this embodiment of the methods of the present invention, the collagen fibers are dried.

Brief Summary Paragraph Right (39):
It is further contemplated that the biocompatible collagen be comprised of additional compounds, including but not limited to antimicrobials, antivirals, growth factors, anti-dehydration compounds, antiseptics, or other compounds suitable for biomedical and/or veterinary uses.

Detailed Description Paragraph Right (18):
As used herein, the term "dehydrated collagen" refers to a collagen sample that has been dehydrated using any method commonly known in the art. In preferred embodiments, dehydrated collagen is produced by lyophilization or desiccation.

Detailed Description Paragraph Right (44):
First, 100 g of clean, dehaired bovine hide obtained from a local slaughterhouse was chopped or sliced into pieces of approximately 1 cm thickness and approximately 10 cm long. The cut slices were washed in three liters of a wash buffer containing double distilled water containing 30 g NaCl, 3 g ZnCl, 4.5 g methyl paraben, 0.9 g propyl paraben. In some experiments, the wash buffer contained 30.0 g NaCl, 3 g ZnCl, 4.5 g methyl paraben, and 1.5 g trichlorophenate or 3 g hypochlorite. No differences were observed in the purified collagen preparations using these different wash buffers. Washing was conducted at 5.degree.-15.degree. C. for 6-8 hours under constant agitation using a Rotator (Model #4140, American). The wash procedure was repeated twice, with the fluid being decanted from the solids after each wash. The repeated wash procedure removed most of the albumins and globulins that usually constitute approximately 1-5% of the dry weight of the sample.

Detailed Description Paragraph Right (51):
In this Example, the "Twice-Treatment Process.TM." (TTP.TM.) is described. In this Example, type I collagen was purified from dehaired bovine hide. First, 100 g of clean, dehaired bovine hide obtained from a local slaughterhouse was chopped or sliced into pieces of approximately 1 cm thickness and approximately 10 cm long. The cut slices were washed in three liters of a wash buffer containing double distilled water containing 30 g NaCl, 3 g ZnCl, 4.5 g methyl paraben, 0.9 g propyl paraben. In some experiments, the wash buffer contained 30.0 g NaCl, 3 g ZnCl, 4.5 g methyl paraben, and 1.5 g trichlorophenate or 3 g hypochlorite. No differences were observed in the purified collagen preparations using these different wash buffers. Washing was conducted at 5.degree.-15.degree. C. for 6-8 hours under constant agitation using a rotator (Model #4140, American) at 80-100 rpm. The wash procedure was repeated twice, with centrifugation at 5,000.times.g for 15 minutes, and decanting of the supernatant each time. The repeated wash procedure removed most of the albumins and globulins that usually constitute approximately 1-5% of the dry weight of the sample.

Detailed Description Paragraph Right (75):
The delipidated and dehydrated type I collagen sample was reconstituted in 0.1M acetic acid, to a concentration of 0.3% (w/v). The purity of the collagen was evaluated by HPLC to determine its amino acid content using a Beckman 6300 amino acid analyzer, according the manufacturer's instructions. The cysteine content of the sample was determined, as was the cysteine content of a collagen preparation that was "less phosphorylated," as described in Example 6. These results indicated that the mole percentage of both the TTP.TM.-treated collagen and "less phosphorylated collagen" was 0.09.

Detailed Description Paragraph Right (77):
The hydroxyproline and hexosamine contents of the delipidated and dehydrated type I collagen, as well as collagen prepared according to the methods described in U.S. Pat. No. 5,374,539, and two phosphorylated collagen samples ("more phosphorylated" and "less phosphorylated") described in Example 6, were tested in the following

experiments.

Detailed Description Paragraph Right (78):
The hydroxyproline content of the delipidated and dehydrated type I collagen, as well as collagen prepared according to the methods described in U.S. Pat. No. 5,374,539, and two phosphorylated collagen samples ("more phosphorylated" and "less phosphorylated") described in Example 6, were estimated using the method described by Stegeman (H. Stegeman, J. Physiol. Chem., 311:41 [19581]). The 0.03M chloramine T used in this Example was prepared by added 0.845 g chloramine T to 100 ml of a buffer comprised of 20 ml water, 30 ml propanol, and 50 ml citrate-acetate buffer, pH 6. The citrate-acetate buffer (pH 6) was prepared by adding 50 g citric acid (1 H.sub.2 O), 12 ml glacial acetic acid, 120 g sodium acetate (3 H.sub.2 O), and 34 g NaOH to 500 ml water. The volume was adjusted to 1 liter, and the pH was adjusted to 6. Perchloric acid (3M) was prepared in 25.5 ml 70% HClO.sub.4, and the volume adjusted to 100 ml in water. The hydroxyproline stock was comprised of 5 .mu.g hydroxyproline per ml water. The hydroxyproline standards were prepared by adding 20 mg hydroxyproline per 100 ml water and 0.05 ml concentrated HCl.

Detailed Description Paragraph Right (80):
The total hexosamine concentration of the delipidated and dehydrated type I collagen, as well as collagen prepared according to the methods described in U.S. Pat. No. 5,374,539, and two phosphorylated collagen samples ("more phosphorylated" and "less phosphorylated") described in Example 6, were also estimated, using the method described by Blumenkrantz et al (N. Blumenkrantz and G. Absoe-Hansen, Clin. Biochem., 9:269 [1976]).

Detailed Description Paragraph Right (103):
The entire skin flap, including the grafted collagen was removed from each of the animals from the surgical site, and fixed in 70% alcohol, dehydrated, and embedded in wax, using methods known in the art. Embedded samples were cut into sections 5 microns in thickness, and stained with Goldner's one-step trichrome stain (OST), and hematoxylin and eosin (H & E), using methods known in the art.

Detailed Description Paragraph Right (127):
In this Example, adult male Sprague-Dawley rats were used. Circular regions (2 cm) of full-thickness skin were surgically removed from the backs of anesthetized the animals, as described in Example 10. The exposed areas were aseptically covered with the collagen preparations (i.e., grafted) described in Example 13. After 3 weeks, the animals were sacrificed and the grafts were surgically removed, fixed in 70% alcohol, dehydrated and embedded in paraffin, using methods commonly known in the art. Sections of 5 micron in thickness were cut, and stained with OST and H & E, using methods commonly known in the art.

CLAIMS:

5. The method of claim 4, further comprising the steps of f) compressing said delipidated collagen to produce compressed collagen; g) dehydrating said compressed collagen to produce dehydrated collagen; and h) dispersing and drying said dehydrating collagen to form collagen fibers.

15. The method of claim 10, further comprising the steps of compressing said delipidated collagen to produce compressed collagen; dehydrating said compressed collagen to produce dehydrated collagen; and dispersing and drying said dehydrating collagen to form collagen fibers, prior to phosphorylating said delipidated collagen.

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L7: Entry 5 of 11 File: USPT Sep 29, 1998

DOCUMENT-IDENTIFIER: US 5814328 A
TITLE: Preparation of collagen using papain and a reducing agent

Abstract Paragraph Left (1):
Purified collagen is produced by contacting a collagen sample with a first proteolytic enzyme, a reducing agent and a second proteolytic enzyme. Preferably, the first and second proteolytic enzymes are papain and the reducing agent is sodium sulfide, dithiothreitol, glutathionine or sodium borohydride. The purified collagen may be contacted with a delipidation agent such as chloroform or methanol and with a phosphorylation agent such as sodium trimetaphosphate to form a biocompatible collagen. Prior to phosphorylation, the delipidated collagen may be treated by compressing, dehydrating, dispersing and drying to form collagen fibers. Also, prior to phosphorylation, the delipidated collagen may be subjected to filter-sterilizing. De-epithelializing of the collagen may carried out prior to treating with the first proteolytic enzyme. The purified and biocompatible collagen may be used in transplantation or hemostasis, and may be provided with compounds such as antimicrobials, antivirals, growth factors and other compounds suitable for biomedical use.

Brief Summary Paragraph Right (15):
Collagen preparations are typically prepared from skin, tendons (e.g., bovine Achilles, tail, and extensor tendons), hide or other animal parts, by procedures involving acid and/or enzyme extraction. Basically, collagen preparation methods involve purification of collagen by extraction with diluted organic acids, precipitation with salts, optional gelation and/or lyophilization, tangential filtration etc. After separating facia, fat and the impurities, the tissue is subjected to moderate digestion with proteolytic enzymes, such as pepsin, then the collagen is precipitated at a neutral pH, redissolved and the residual impurities precipitated at an acid pH. The tissue is then digested with a strong alkali and then exposed to acid to facilitated swelling. The collagen fibers are then precipitated with salts or organic solvents, and dehydrating the collagen fibers. (See e.g., U.S. Pat. No. 5,028,695, herein incorporated by reference). Eventually the extracted collagen can be converted into a finely divided fibrous collagen by treating water-wet collagen with acetone to remove water, centrifuging to obtain the solid mass of collagen and deaggregating the collagen during drying. (See e.g., U.S. Pat. No. 4,148,664, herein incorporated by reference). The collagen preparation can then be brought back to a neutral pH and dried in the form of fibers. Completely transparent, physiological and hemocompatible gels, collagen films, and solutions can be prepared. These forms of collagen may then be used in the fabrication of contact lenses and implants.

Brief Summary Paragraph Right (26):
In a particularly preferred embodiment, the method of the present invention further comprises the steps of compressing the delipidated collagen to produce compressed collagen; dehydrating the compressed collagen to produce dehydrated collagen; and dispersing and drying the dehydrating collagen to form collagen fibers. Thus, in this embodiment of the methods of the present invention, the collagen fibers are dried.

Brief Summary Paragraph Right (29):
It is further contemplated that the purified collagen be comprised of additional compounds, including but not limited to antimicrobials, antivirals, growth factors, anti-dehydration compounds, antiseptics, or other compounds suitable for biomedical

and/or veterinary uses.

Brief Summary Paragraph Right (35):
In yet a further embodiment, the method of the present invention further comprises the steps of compressing the delipidated collagen to produce compressed collagen; dehydrating the compressed collagen to produce dehydrated collagen; and dispersing and drying the dehydrating collagen to form collagen fibers. Thus, in this embodiment of the methods of the present invention, the collagen fibers are dried.

Brief Summary Paragraph Right (39):
It is further contemplated that the biocompatible collagen be comprised of additional compounds, including but not limited to antimicrobials, antivirals, growth factors, anti-dehydration compounds, antiseptics, or other compounds suitable for biomedical and/or veterinary uses.

Detailed Description Paragraph Right (18):
As used herein, the term "dehydrated collagen" refers to a collagen sample that has been dehydrated using any method commonly known in the art. In preferred embodiments, dehydrated collagen is produced by lyophilization or desiccation.

Detailed Description Paragraph Right (44):
First, 100 g of clean, dehaired bovine hide obtained from a local slaughterhouse was chopped or sliced into pieces of approximately 1 cm thickness and approximately 10 cm long. The cut slices were washed in three liters of a wash buffer containing double distilled water containing 30 g NaCl, 3 g ZnCl, 4.5 g methyl paraben, 0.9 g propyl paraben. In some experiments, the wash buffer contained 30.0 g NaCl, 3 g ZnCl, 4.5 g methyl paraben, and 1.5 g trichlorophenate or 3 g hypochlorite. No differences were observed in the purified collagen preparations using these different wash buffers. Washing was conducted at 5.degree.-15.degree. C. for 6-8 hours under constant agitation using a Rotator (Model #4140, American). The wash procedure was repeated twice, with the fluid being decanted from the solids after each wash. The repeated wash procedure removed most of the albumins and globulins that usually constitute approximately 1-5% of the dry weight of the sample.

Detailed Description Paragraph Right (51):
In this Example, the "Twice-Treatment Process.TM." (TTP.TM.) is described. In this Example, type I collagen was purified from dehaired bovine hide. First, 100 g of clean, dehaired bovine hide obtained from a local slaughterhouse was chopped or sliced into pieces of approximately 1 cm thickness and approximately 10 cm long. The cut slices were washed in three liters of a wash buffer containing double distilled water containing 30 g NaCl, 3 g ZnCl, 4.5 g methyl paraben, 0.9 g propyl paraben. In some experiments, the wash buffer contained 30.0 g NaCl, 3 g ZnCl, 4.5 g methyl paraben, and 1.5 g trichlorophenate or 3 g hypochlorite. No differences were observed in the purified collagen preparations using these different wash buffers. Washing was conducted at 5.degree.-15.degree. C. for 6-8 hours under constant agitation using a rotator (Model #4140, American) at 80-100 rpm. The wash procedure was repeated twice, with centrifugation at 5,000.times.g for 15 minutes, and decanting of the supernatant each time. The repeated wash procedure removed most of the albumins and globulins that usually constitute approximately 1-5% of the dry weight of the sample.

Detailed Description Paragraph Right (75):
The delipidated and dehydrated type I collagen sample was reconstituted in 0.1M acetic acid, to a concentration of 0.3% (w/v). The purity of the collagen was evaluated by HPLC to determine its amino acid content using a Beckman 6300 amino acid analyzer, according the manufacturer's instructions. The cysteine content of the sample was determined, as was the cysteine content of a collagen preparation that was "less phosphorylated," as described in Example 6. These results indicated that the mole percentage of both the TTP.TM.-treated collagen and "less phosphorylated collagen" was 0.09.

Detailed Description Paragraph Right (77):
The hydroxyproline and hexosamine contents of the delipidated and dehydrated type I collagen, as well as collagen prepared according to the methods described in U.S. Pat. No. 5,374,539, and two phosphorylated collagen samples ("more phosphorylated" and "less phosphorylated") described in Example 6, were tested in the following

experiments.

Detailed Description Paragraph Right (78):
The hydroxyproline content of the delipidated and dehydrated type I collagen, as well as collagen prepared according to the methods described in U.S. Pat. No. 5,374,539, and two phosphorylated collagen samples ("more phosphorylated" and "less phosphorylated") described in Example 6, were estimated using the method described by Stegeman (H. Stegeman, J. Physiol. Chem., 311:41 [19581]). The 0.03M chloramine T used in this Example was prepared by added 0.845 g chloramine T to 100 ml of a buffer comprised of 20 ml water, 30 ml propanol, and 50 ml citrate-acetate buffer, pH 6. The citrate-acetate buffer (pH 6) was prepared by adding 50 g citric acid (1 H.sub.2 O), 12 ml glacial acetic acid, 120 g sodium acetate (3 H.sub.2 O), and 34 g NaOH to 500 ml water. The volume was adjusted to 1 liter, and the pH was adjusted to 6. Perchloric acid (3M) was prepared in 25.5 ml 70% HClO.sub.4, and the volume adjusted to 100 ml in water. The hydroxyproline stock was comprised of 5 .mu.g hydroxyproline per ml water. The hydroxyproline standards were prepared by adding 20 mg hydroxyproline per 100 ml water and 0.05 ml concentrated HCl.

Detailed Description Paragraph Right (80):
The total hexosamine concentration of the delipidated and dehydrated type I collagen, as well as collagen prepared according to the methods described in U.S. Pat. No. 5,374,539, and two phosphorylated collagen samples ("more phosphorylated" and "less phosphorylated") described in Example 6, were also estimated, using the method described by Blumenkrantz et al (N. Blumenkrantz and G. Absoe-Hansen, Clin. Biochem., 9:269 [1976]).

Detailed Description Paragraph Right (103):
The entire skin flap, including the grafted collagen was removed from each of the animals from the surgical site, and fixed in 70% alcohol, dehydrated, and embedded in wax, using methods known in the art. Embedded samples were cut into sections 5 microns in thickness, and stained with Goldner's one-step trichrome stain (OST), and hematoxylin and eosin (H & E), using methods known in the art.

Detailed Description Paragraph Right (127):
In this Example, adult male Sprague-Dawley rats were used. Circular regions (2 cm) of full-thickness skin were surgically removed from the backs of anesthetized the animals, as described in Example 10. The exposed areas were aseptically covered with the collagen preparations (i.e., grafted) described in Example 13. After 3 weeks, the animals were sacrificed and the grafts were surgically removed, fixed in 70% alcohol, dehydrated and embedded in paraffin, using methods commonly known in the art. Sections of 5 micron in thickness were cut, and stained with OST and H & E, using methods commonly known in the art.

CLAIMS:

5. The method of claim 4, further comprising the steps of f) compressing said delipidated collagen to produce compressed collagen; g) dehydrating said compressed collagen to produce dehydrated collagen; and h) dispersing and drying said dehydrating collagen to form collagen fibers.

15. The method of claim 10, further comprising the steps of compressing said delipidated collagen to produce compressed collagen; dehydrating said compressed collagen to produce dehydrated collagen; and dispersing and drying said dehydrating collagen to form collagen fibers, prior to phosphorylating said delipidated collagen.

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L7: Entry 3 of 11 File: USPT Oct 3, 2000

DOCUMENT-IDENTIFIER: US 6127143 A
TITLE: Preparation of purified and biocompatible collagen using two proteolytic enzyme treatments and a reducing agent

Abstract Paragraph Left (1):
Purified collagen is produced by a method containing steps of contacting a collagen sample with a first proteolytic enzyme followed by contacting with a reducing agent and a second proteolytic enzyme. Preferably, the first and second proteolytic enzymes are papain and the reducing agent is sodium sulfide, dithiothreitol, glutathionine or sodium borohydride. A biocompatible collagen is prepared by contacting the purified collagen with a delipidation agent such as chloroform or methanol to produce delipidated collagen, and then contacting the delipidated collagen with a phosphorylation agent such as sodium trimetaphosphate. Prior to phosphorylation, the delipidated collagen may be treated by compressing, dehydrating, dispersing and drying to form collagen fibers. Also, prior to phosphorylation, the delipidated collagen may be treated by filter-sterilizing. De-epithelializing of the collagen may carried out prior to treating with the first proteolytic enzyme. The purified and biocompatible collagen may be used in transplantation or hemostasis, and may be provided with compounds such as antimicrobials, antivirals, growth factors and other compounds suitable for biomedical use.

Brief Summary Paragraph Right (16):
Collagen preparations are typically prepared from skin, tendons (e.g., bovine Achilles, tail, and extensor tendons), hide or other animal parts, by procedures involving acid and/or enzyme extraction. Basically, collagen preparation methods involve purification of collagen by extraction with diluted organic acids, precipitation with salts, optional gelation and/or lyophilization, tangential filtration etc. After separating facia, fat and the impurities, the tissue is subjected to moderate digestion with proteolytic enzymes, such as pepsin, then the collagen is precipitated at a neutral pH, redissolved and the residual impurities precipitated at an acid pH. The tissue is then digested with a strong alkali and then exposed to acid to facilitated swelling. The collagen fibers are then precipitated with salts or organic solvents, and dehydrating the collagen fibers. (See e.g., U.S. Pat. No. 5,028,695, herein incorporated by reference). Eventually the extracted collagen can be converted into a finely divided fibrous collagen by treating water-wet collagen with acetone to remove water, centrifuging to obtain the solid mass of collagen and deaggregating the collagen during drying. (See e.g., U.S. Pat. No. 4,148,664, herein incorporated by reference). The collagen preparation can then be brought back to a neutral pH and dried in the form of fibers. Completely transparent, physiological and hemocompatible gels, collagen films, and solutions can be prepared. These forms of collagen may then be used in the fabrication of contact lenses and implants.

Brief Summary Paragraph Right (27):
In a particularly preferred embodiment, the method of the present invention further comprises the steps of compressing the delipidated collagen to produce compressed collagen; dehydrating the compressed collagen to produce dehydrated collagen; and dispersing and drying the dehydrating collagen to form collagen fibers. Thus, in this embodiment of the methods of the present invention, the collagen fibers are dried.

Brief Summary Paragraph Right (30):
It is further contemplated that the purified collagen be comprised of additional

compounds, including but not limited to antimicrobials, antivirals, growth factors, anti-dehydration compounds, antiseptics, or other compounds suitable for biomedical and/or veterinary uses.

Brief Summary Paragraph Right (36):
In yet a further embodiment, the method of the present invention further comprises the steps of compressing the delipidated collagen to produce compressed collagen; dehydrating the compressed collagen to produce dehydrated collagen; and dispersing and drying the dehydrating collagen to form collagen fibers. Thus, in this embodiment of the methods of the present invention, the collagen fibers are dried.

Brief Summary Paragraph Right (40):
It is further contemplated that the biocompatible collagen be comprised of additional compounds, including but not limited to antimicrobials, antivirals, growth factors, anti-dehydration compounds, antiseptics, or other compounds suitable for biomedical and/or veterinary uses.

Detailed Description Paragraph Right (19):
As used herein, the term "dehydrated collagen" refers to a collagen sample that has been dehydrated using any method commonly known in the art. In preferred embodiments, dehydrated collagen is produced by lyophilization or desiccation.

Detailed Description Paragraph Right (46):
First, 100 g of clean, dehaired bovine hide obtained from a local slaughterhouse was chopped or sliced into pieces of approximately 1 cm thickness and approximately 10 cm long. The cut slices were washed in three liters of a wash buffer containing double distilled water containing 30 g NaCl, 3 g ZnCl, 4.5 g methyl paraben, 0.9 g propyl paraben. In some experiments, the wash buffer contained 30.0 g NaCl, 3 g ZnCl, 4.5 g methyl paraben, and 1.5 g trichlorophenate or 3 g hypochlorite. No differences were observed in the purified collagen preparations using these different wash buffers. Washing was conducted at 5-15.degree. C. for 6-8 hours under constant agitation using a Rotator (Model #4140, American). The wash procedure was repeated twice, with the fluid being decanted from the solids after each wash. The repeated wash procedure removed most of the albumins and globulins that usually constitute approximately 1-5% of the dry weight of the sample.

Detailed Description Paragraph Right (53):
In this Example, the "Twice-Treatment Process.TM." (TTP.TM.) is described. In this Example, type I collagen was purified from dehaired bovine hide. First, 100 g of clean, dehaired bovine hide obtained from a local slaughterhouse was chopped or sliced into pieces of approximately 1 cm thickness and approximately 10 cm long. The cut slices were washed in three liters of a wash buffer containing double distilled water containing 30 g NaCl, 3 g ZnCl, 4.5 g methyl paraben, 0.9 g propyl paraben. In some experiments, the wash buffer contained 30.0 g NaCl, 3 g ZnCl, 4.5 g methyl paraben, and 1.5 g trichlorophenate or 3 g hypochlorite. No differences were observed in the purified collagen preparations using these different wash buffers. Washing was conducted at 5-15.degree. C. for 6-8 hours under constant agitation using a rotator (Model #4140, American) at 80-100 rpm. The wash procedure was repeated twice, with centrifugation at 5,000.times.g for 15 minutes, and decanting of the supernatant each time. The repeated wash procedure removed most of the albumins and globulins that usually constitute approximately 1-5% of the dry weight of the sample.

Detailed Description Paragraph Right (78):
The delipidated and dehydrated type I collagen sample was reconstituted in 0.1 M acetic acid, to a concentration of 0.3% (w/v). The purity of the collagen was evaluated by HPLC to determine its amino acid content using a Beckman 6300 amino acid analyzer, according the manufacturer's instructions. The cysteine content of the sample was determined, as was the cysteine content of a collagen preparation that was "less phosphorylated," as described in Example 6. These results indicated that the mole percentage of both the TTP.TM.-treated collagen and "less phosphorylated collagen" was 0.09.

Detailed Description Paragraph Right (80):
The hydroxyproline and hexosamine contents of the delipidated and dehydrated type I collagen, as well as collagen prepared according to the methods described in U.S.

Pat. No. 5,374,539, and two phosphorylated collagen samples ("more phosphorylated" and "less phosphorylated") described in Example 6, were tested in the following experiments.

Detailed Description Paragraph Right (81):
The hydroxyproline content of the delipidated and dehydrated type I collagen, as well as collagen prepared according to the methods described in U.S. Pat. No. 5,374,539, and two phosphorylated collagen samples ("more phosphorylated" and "less phosphorylated") described in Example 6, were estimated using the method described by Stegeman (H. Stegeman, J. Physiol. Chem., 311:41 [1958]). The 0.03 M chloramine T used in this Example was prepared by added 0.845 g chloramine T to 100 ml of a buffer comprised of 20 ml water, 30 ml propanol, and 50 ml citrate-acetate buffer, pH 6. The citrate-acetate buffer (pH 6) was prepared by adding 50 g citric acid (1 H.sub.2 O), 12 ml glacial acetic acid, 120 g sodium acetate (3 H.sub.2 O), and 34 g NaOH to 500 ml water. The volume was adjusted to 1 liter, and the pH was adjusted to 6. Perchloric acid (3 M) was prepared in 25.5 ml 70% HClO.sub.4, and the volume adjusted to 100 ml in water. The hydroxyproline stock was comprised of 5 .mu.g hydroxyproline per ml water. The hydroxyproline standards were prepared by adding 20 mg hydroxyproline per 100 ml water and 0.05 ml concentrated HCl.

Detailed Description Paragraph Right (83):
The total hexosamine concentration of the delipidated and dehydrated type I collagen, as well as collagen prepared according to the methods described in U.S. Pat. No. 5,374,539, and two phosphorylated collagen samples ("more phosphorylated" and "less phosphorylated") described in Example 6, were also estimated, using the method described by Blumenkrantz et al. (N. Blumenkrantz and G. Absoe-Hansen, Clin. Biochem., 9:269 [1976]).

Detailed Description Paragraph Right (107):
The entire skin flap, including the grafted collagen was removed from each of the animals from the surgical site, and fixed in 70% alcohol, dehydrated, and embedded in wax, using methods known in the art. Embedded samples were cut into sections 5 microns in thickness, and stained with Goldner's one-step trichrome stain (OST), and hematoxylin and eosin (H & E), using methods known in the art.

Detailed Description Paragraph Right (132):
In this Example, adult male Sprague-Dawley rats were used. Circular regions (2 cm) of full-thickness skin were surgically removed from the backs of anesthetized the animals, as described in Example 10. The exposed areas were aseptically covered with the collagen preparations (i.e., grafted) described in Example 13. After 3 weeks, the animals were sacrificed and the grafts were surgically removed, fixed in 70% alcohol, dehydrated and embedded in paraffin, using methods commonly known in the art. Sections of 5 micron in thickness were cut, and stained with OST and H & E, using methods commonly known in the art.

CLAIMS:

7. The method of claim 6, further comprising the steps of g) compressing said delipidated collagen to produce compressed collagen; h) dehydrating said compressed collagen to produce dehydrated collagen; and i) dispersing and drying said dehydrating collagen to form collagen fibers.

20. The method of claim 15, further comprising the steps of compressing said delipidated collagen to produce compressed collagen; dehydrating said compressed collagen to produce dehydrated collagen; and dispersing and drying said dehydrating collagen to form collagen fibers, prior to phosphorylating said delipidated collagen.

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L1: Entry 3 of 13 File: USPT Jan 21, 1986

DOCUMENT-IDENTIFIER: US 4565700 A
TITLE: Process for preparation of substance having hypotensive component

Detailed Description Paragraph Right (6):
To 5 kg of a degummed oil from extracted soybean oil was added 7.50 cc of a 2% aqueous solution of lactic acid, and the mixture was stirred at 40.degree. C. for 10 minutes. The aqueous layer was separated and concentrated at 70.degree. C. by a flash evaporator, and the concentrate was subjected to evaporation to dryness. Then, the solid was extracted two times with 50 cc of a 75% aqueous solution of ethanol, and the extract was recovered by filtration and concentrated by a flash evaporator so that the volume was reduced to 1/10. The concentrate was dried at a temperature lower than 40.degree. C. by a vacuum drier to obtain 0.54 g of a solid having a light yellowish brown color. The results of the animal test are shown in Curve 3 of FIG. 2.

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L13: Entry 3 of 4 File: DWPI Oct 5, 1983

DERWENT-ACC-NO: 1984-030485
DERWENT-WEEK: 198406
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TITLE: High purity protein isolate prodn. from plant material - by extn. with salt soln., ultrafiltration and dialysis

INVENTOR: GASSMANN, B; KROECK, R ; KROLL, J

PATENT-ASSIGNEE:

ASSIGNEE	CODE
AKAD WISSENSCHAFTEN DDR	DEAK

PRIORITY-DATA: 1982DD-0237034 (January 28, 1982)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
DD 202800 A	October 5, 1983		000	

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
DD 202800A	January 28, 1982	1982DD-0237034	

INT-CL (IPC): A23J 1/14

ABSTRACTED-PUB-NO: DD 202800A
BASIC-ABSTRACT:

Process comprises first extracting with aq. 0.5-7 (3-5)% salt (esp. NaCl) soln., sepg. insolubles by decanting, centrifuging or filtration, then opt. purifying the extract by pptn. followed by centrifuging or filtering. The new feature is that the soluble protein fraction is both sepd. and purified by ultrafiltration of the extract, then eliminating NaCl and other low mol. wt. materials from the retentate by dialysis against water. The dialysate is then dried, opt. after sepn. of dissolved and suspended proteins.

The extract is pref. ultrafiltered to protein concn. 1-8(3-6)% and dialysis (pref. with continuous water renewal), is to NaCl content 0.01-0.5 (0.05)%.

The method is esp. applied to seeds contg. globulins and albumins, e.g. sunflower or rape. It provides almost quantitative recovery of high-purity proteins under mild conditions and without requiring pptn. The prods. are fully functional, esp. they retain the foaming and emulsifying properties.

TITLE-TERMS: HIGH PURE PROTEIN ISOLATE PRODUCE PLANT MATERIAL EXTRACT SALT SOLUTION ULTRAFILTER DIALYSE

DERWENT-CLASS: D13

CPI-CODES: D03-F01;

UNLINKED-DERWENT-REGISTRY-NUMBERS: 1706U

SECONDARY-ACC-NO:

WEST

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L5: Entry 59 of 69 File: DWPI Mar 27, 1995

DERWENT-ACC-NO: 1995-343064
DERWENT-WEEK: 199544
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TITLE: A method of obtaining peroxidase(s) - from homogenised Artemesia plant tissue by sodium chloride extraction, ion exchange chromatography and gel filtration

INVENTOR: ASKAROVA, M A; KUNAEVA, R M ; MUKHAMEDZHANOV, B G

PATENT-ASSIGNEE:

ASSIGNEE	CODE
AS KAZA MOL BIOLOG BIOCHEM INST	AKMOR

PRIORITY-DATA: 1992SU-5021066 (January 8, 1992)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
RU 2031946 C1	March 27, 1995		004	C12N009/08

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
RU 2031946C1	January 8, 1992	1992SU-5021066	

INT-CL (IPC): C12 N 9/08

ABSTRACTED-PUB-NO: RU 2031946C

BASIC-ABSTRACT:

A method of obtaining peroxidase by homogenisation of Artemesia plant tissue, extn. of the enzyme with an aq. soln. of common salt, fractionation with ammonium sulphate, and subsequent ion exchange chromatography (IEC) and gel filtration is new. A capsule Artemesia culture is used as the plant tissue, and the enzyme is extracted with 0.2-0.5 molar NaCl soln. The soln. thus obtained is subjected to (IEC), with successive purification on DEAE-cellulose and KM-cellulose, followed by gel filtration.

ADVANTAGE - The method gives peroxidase in increased yield and with higher activity than with previous methods. PEROXIDASE ACTIVITY - The specific peroxidase activity after gel filtration on sephadex T-50 was 386 micromole/g x 103, as against 9.25 for the prototype.

CHOSEN-DRAWING: Dwg.0/0

TITLE-TERMS: METHOD OBTAIN PEROXIDASE HOMOGENISE PLANT TISSUE SODIUM CHLORIDE EXTRACT ION EXCHANGE CHROMATOGRAPHY GEL FILTER

DERWENT-CLASS: A97 D16

CPI-CODES: A03-A04A; A03-A05; A12-L04A; A12-M05; A12-W11L; D05-C03B; D05-H13;

UNLINKED-DERWENT-REGISTRY-NUMBERS: 1704S; 1706S

ENHANCED-POLYMER-INDEXING:
Polymer Index [1.1] 017 ; G3689 G3678 G3634 D01 D03 D11 D10 D23 D22 D42 F24 F34 H0293 P0599 G3623 Polymer Index [1.2] 017 ; G3634*R D01 D03 D11 D10 D23 D22 D31 D42 F24 F34

H0293 P0599 G3623 Polymer Index [1.3] 017 ; ND01 ; Q9999 Q7807 Q7794 ; Q9999 Q7772 ; Q9999 Q8082

SECONDARY-ACC-NO:
CPI Secondary Accession Numbers: C1995-150959

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L5: Entry 64 of 69 File: DWPI Jun 30, 1984

DERWENT-ACC-NO: 1985-024766
DERWENT-WEEK: 198504
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TITLE: Recovery of acidic desoxy-ribonuclease from liver tissue - by acid extn., salting out, desalination and chromatography on phospho-cellulose and then on anion exchanger

INVENTOR: FEDOSOV, Y U V; KOZHEMYAKO, V B ; RASSKAZOV, V A

PATENT-ASSIGNEE:

ASSIGNEE	CODE
AS USSR FAR E OCEAN	ASFAR

PRIORITY-DATA: 1982SU-3574600 (December 14, 1982)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
SU 1100308 A	June 30, 1984		004	

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
SU 1100308A	December 14, 1982	1982SU-3574600	

INT-CL (IPC): C12N 9/16

ABSTRACTED-PUB-NO: SU 1100308A
BASIC-ABSTRACT:

Acid desoxyribonuclease is obtd. by: extracting liver tissue with aq. acid; fractionating the extract with (NH4)2SO4; desalinating; chromatographing an phosphocellulose, eluting with buffer at pH 7.1-7.2; and chromatographing on an anion-exchanger, eluting with the same buffer contg. 0.3-0.35M NaCl. Pref. anion exchangers are DEAE-cellulose, DEAE-sephadex, TEAE-cellulose and AE-cellulose.

ADVANTAGE - The method is simple and produces a good yield.

CHOSEN-DRAWING: Dwg.0/0

TITLE-TERMS: RECOVER ACIDIC DESOXY RIBONUCLEASE LIVER TISSUE ACID EXTRACT SALT DESALINATE CHROMATOGRAPHY PHOSPHO CELLULOSE ANION EXCHANGE

DERWENT-CLASS: A91 B04 D16

CPI-CODES: A03-A04A; A10-E08C; A10-E20; A12-M05; B04-B02C3; D05-A02;

CHEMICAL-CODES:

Chemical Indexing M1 *01*
Fragmentation Code
M423 M720 M903 N134 N136 N161 N421 N425 N512 V802 V815